DNA 3500 CE Databasing

A. SCOPE

Amplified DNA fragments are separated by capillary electrophoresis (CE) on a 3500xL Genetic Analyzer. Fluorescence detection is accomplished by utilizing a single-line solid-state laser to excite fluorescent dyes and a CCD camera that records the fluorescence emitted from samples within each of the twenty-four capillaries. The resulting data is subsequently analyzed using GeneMapper ID-X software. The fragment size is automatically assigned based on an internal size standard, which is co-electrophoresed with each sample. Alleles are assigned based on comparison of the fragment size of the unknown peak to that of the allelic ladder.

For the purpose of this protocol, the following definitions apply:

Injection: all samples that are separated via CE at a single time. Thus for one injection 24 samples can be separated at a single time.

Run: all samples that were defined on a single plate and loaded onto the instrument at a single time for separation, regardless of the number of injections utilized to separate each sample contained on the plate. Thus, a run could contain up to 96 samples (including ladders and controls) for each separation on a single plate.

B. QUALITY CONTROL

B.1. Protective gloves and a lab coat must be worn when performing this procedure to prevent contamination.

B.2. See DOC ID 1835 to determine reagent expiration dates.

B.3. Do not clean any components or accessories of the 3500xL with bleach or ethanol. Clean with deionized water.

B.4. Hi-Di Formamide: To prevent repeated thawing and re-freezing of formamide, aliquot formamide into approximately 500 and 1000 µL volumes after initially thawing the 25 mL bottle. Appropriately discard any unused aliquot of thawed formamide.

C. SAFETY

C.1. Hi-Di Formamide: exposure causes eye, skin, and respiratory tract irritation. It is also a possible developmental and birth defect hazard.

C.2. All appropriate SDS sheets must be read prior to performing this procedure.

C.3. Protective gloves, a lab coat and eye protection (e.g. safety glasses or a face shield) must be worn at all times when performing this procedure.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1. GlobalFiler Allelic Ladder

D.2. 3500 Performance Optimized Polymer (POP-4 polymer)

D.3. Prefilled 3500xL Genetic Analyzer Anode and Cathode containers

D.4. GS-600 LIZ Size Standard

D.5. Hi-Di Formamide

E. EQUIPMENT & SUPPLIES

E.1. Equipment

E.1.1 AB 3500xL Genetic Analyzer (instrument, computer and appropriate software)
E.1.2 AB 36cm 24 capillary array
E.1.3 AB 3500xL Genetic Analyzer sample septa
E.1.4 Thermal cycler
E.1.5 Pipettes
E.1.6 Vortexer
E.1.7 Frozen plate block
E.1.8 Decapper
E.1.9 96-well plate retainer and base
E.1.10 96-well plate centrifuge

E.2. Supplies

E.2.1 Pipette tips
E.2.2 Gloves
E.2.3 Lab coat
E.2.4 Eye protection (e.g. safety glasses, face shield)
E.2.5 3500xL template (Save as .txt file before importing on 3500xL)

F. PROCEDURES

F.1. **GENERAL**

F.1.1 The AB 3500 – User Guide must be referenced for all ordinary operations and troubleshooting of the 3500xL instrumentation.

F.1.2 All maintenance (daily (should be read as "before each run on an "as needed" basis); weekly; monthly; quarterly; "as needed") will be performed according to the guidance outlined from Page 229 onwards of the AB 3500 – User Guide.

- **Note**: The "Wash Pump and Channels Wizard" in the weekly list need only be performed if the instrument has not been in use during the prior week.

F.1.3 Start the CE system in the following order:

F.1.3.1. Computer: Logon as INSTR-USER (username and password are INSTR-USER)

F.1.3.2. Turn on the 3500xL instrument: Wait for the status light to turn green

F.1.3.3. Launch the 3500 data-collection software

F.1.3.3.1. User = **Administrator** / Password = **Administrator1**

F.1.4 From the Dashboard of the data-collection software, the user must use the Wizard button to perform all necessary reagent / capillary array changes; water washes; long-term instrument shutdown and start-ups.

F.1.5 If a new capillary array needs to be installed on the instrument, a spatial calibration must be completed prior to running samples on the instrument. Follow the instruction from Page 99 onwards of the AB 3500 – User Guide to perform a spatial calibration.

F.1.6 A GlobalFiler spectral calibration must also be performed in accordance with the instructions given from Page 103 onwards of the AB 3500 – User Guide.

F.2. CREATING A PLATE DOCUMENT

F.2.1 Complete the 3500xL Database Injection List Template document in the following manner:

F.2.1.1. Enter the name of your plate in cell A4

F.2.1.2. Enter your initials in cell F4

F.2.1.3. Enter sample names in column B from cell B8 onwards.

F.2.1.4. Ladders must remain in at least the well positions: A01; E06; D08; H11

F.2.1.4.1. If only 1 injection needed a second ladder must be added to the injection list

F.2.1.5. Ensure the "Sample Type" in Column F is appropriate for all samples

F.2.1.6. Delete entirely unused injections (groups of 24 only)

F.2.1.7. OPTIONAL: To analyze samples in the order set up on the plate, fill the comments column in numerical order. Numbers 1 -9 must be entered as 01, 02, 03 etc.

F.2.1.8. Save the document as a ".txt" file on to a removable storage device

F.2.2 Set up the CE plate with the samples in the appropriate position by utilizing the epMotion post-amp platform.

F.3. IMPORTING A PLATE DOCUMENT

From the data-collection software:

F.3.1 Choose "Import" from the library menu

F.3.2 Locate and double-click on the appropriate .txt file from the storage device

F.4. GLOBALFILER SAMPLE PREPARATION FOR CE

F.4.1 Preparation of a 3500xL CE plate will be done using the post-amp epMotion according to the relevant protocol.

F.4.2 If the epMotion is unavailable, then the CE plate may be manually setup according to the following:

F.4.3 Combine the necessary amount of formamide and GS 600 LIZ size standard in a microcentrifuge tube as follows:

(Number of samples + 2) x 9.6 µL formamide

(Number of samples + 2) x 0.4 µL GS 600 LIZ

***Note**: It is recommended that enough volume for additional samples be included in the calculation to account for volume lost in pipetting; therefore, depending on the number of samples, more than two extra samples can be incorporated into the above listed calculation.*

F.4.4 Vortex and spin the mixture briefly in a microcentrifuge.

F.4.5 Aliquot 10 µL of the formamide/GS 600 LIZ master mix into each plate well.

F.4.6 Add 1 µL of PCR product or allelic ladder to each plate well. Cover the plate with rubber septa.

F.4.7 Centrifuge the plate briefly.

F.4.8 Heat the samples in a thermal cycler for three minutes at 95°C to denature.

F.4.9 Snap-cool immediately for a minimum of three minutes in frozen plate holder.

F.4.10 Place the plate into the plate base and centrifuge briefly.

F.4.11 Secure sample plate into the base with a plastic retainer clip.

F.5. 3500xL RUN

F.5.1 Choose "Edit Existing Plate" from the dashboard

F.5.2 In the 3 boxes at the bottom of the dashboard choose, or add from library (and click the checkbox):

F.5.2.1. Assay: Database OSR

F.5.2.2. File Name: Database

F.5.2.3. Results Group: Database

F.5.3 Locate and double click the appropriate plate name from the list to open the plate information. Before proceeding to the next step, you **must** highlight the entire plate by clicking the white box in the top left corner of the plate map.

F.5.4 Place the prepared CE plate in a 3500 plate base and cover with a 3500 plate retainer and then seat the plate in the "A" or "B" position on the instrument platform.

F.5.5 Choose the command to: "Link Plate For Run"

F.5.5.1. From here you may edit the injection list, or start the run by choosing the button commands at the bottom of the dashboard.

F.5.6 After initial review of the completed run, reinjections may be deemed necessary.

F.5.6.1. Choose the wells that require reinjection (no need to choose any ladders unless they are of concern) from the plate map.

F.5.6.2. Click the "Reinject" command at the bottom right of the plate map.

F.5.6.2.1. Be sure to use the "existing protocol" when prompted

F.5.7 When the run is complete, terminate the injection list and unlink the plate

F.5.8 The data is stored in the results forlder of the D: drive on the CE's CPU. The analyst must ensure a copy of the data is placed on the "I" drive. The data from the first analysis of any convicted offender sample should be stored under K:\Division\DNA\CODIS\Analysis.

G. INTERPRETATION GUIDELINES

G.1. See DOC ID 12628 (GlobalFiler interpretation guidelines).

H. REFERENCES

H.1. AB 3500 - User Guide

H.2. AB 3500 Data Collection Software v4 - User Bulletin A